

Docket No.: 0649-1217PUS1  
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

---

In re Patent Application of:  
Toshihiro MORI et al.

Application No.: 10/568,101

Confirmation No.: 7811

Filed: February 13, 2006

Art Unit: 1637

For: METHOD FOR ISOLATING AND  
PURIFYING A NUCLEIC ACID

---

Examiner: C. B. Wilder.

**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Toshihiro Mori, declare the following:

I am a citizen of Japan residing at 577, Ushijima, Kaisei-machi, Kanagawa, Japan.

I am a named Inventor of the present application and have read and understand the specification and claims to the above-identified application and the outstanding Office Action of October 31, 2007. I have also read and considered the references cited therein as the basis of the obviousness rejection under 35 U.S.C. § 103(a), including: Su, WO 97/08547, Su, U.S. Patent No. 5,804,684 and Kappel et al., U.S. Patent Application Publication No. 20040259162.

I hereby declare and state that I received a Bachelor degree of pharmaceutical sciences from Tohoku University on March 1987 and that I have been employed as a worker in research by Fuji Photo Film Co. Ltd. (now FUJIFILM Corporation) Since April 1987.

I declare that I am at present doing research work on nucleic acid detection in Advanced Research Laboratory of said company.

As objective evidence of unexpected results weighing against the finding of obviousness, I performed, or had performed under my direct supervision, the following experiments. These experiments compare the presently claimed invention to the most closely related cited references. These experiments provide empirical objective evidence of unexpected results.

### **Comparative Experiments**

#### **(1) Materials and Reagents**

Separation and purification of nucleic acid was performed using the nucleic acid separation and purification unit disclosed at Figures 1 to 6 of the gazette of Japanese Patent Laid-Open No. 2004-180637. Sample, washing solution and distilled water were successively infused from the second opening side and, for each of the infusions, a piston member (plunger) was inserted followed by pushing. Fuji Microfilter FR 250 (manufactured by FUJIFILM Corporation) was used as a solid phase to adsorb the nucleic acid. Pre-treatment solutions (Comparative Example and the present invention) and washing solution were prepared as follows.

#### Pre-treatment Solution (Comparative Example)

Guanidine hydrochloride (manufactured by Life Technology)	382 g
Tris (manufactured by Life Technology)	12.1 g
Triton-X100 (manufactured by ICN Biomedicals, Inc.)	10 g
Distilled water	1,000 ml

Pre-treatment Solution (Present Invention)

Guanidine hydrochloride (manufactured by Life Technology)	382 g
Tris (manufactured by Life Technology)	12.1 g
Triton-X100 (manufactured by ICN Biomedicals, Inc.)	10 g
Antifoaming agent of Table 1	12 g
Distilled water	1,000 ml

Washing Solution

10 mmol/L Tris-HCl, 65% ethanol

(2) Separation and Purification of Nucleic Acid

To 200  $\mu$ l of a human whole blood sample were added 200  $\mu$ l of the Pre-treatment Solution of the Present Invention and 200  $\mu$ l of protease K, followed by incubating at 60°C for 10 minutes. After the incubation, 200  $\mu$ l of ethanol was added thereto, followed by stirring. After stirring, the above liquid was infused into a unit for separation and purification of nucleic acid, whose structure is shown in Figures 1 to 6 of the gazette of Japanese Patent Laid-Open No. 2004-180637. After the infusion, the liquid was pushed out using a piston.

Afterwards, 500  $\mu$ l of the Washing Solution was infused into the unit and the liquid was pushed out using a piston to wash impurities out of the unit. Finally, 200  $\mu$ l of distilled water was infused into the unit and the liquid was pushed out using a piston. This liquid was recovered as a DNA solution.

The same operation for separation and purification of nucleic acid as that in the case of the present invention was carried out except that the Pre-treatment Solution for Comparative Example was substituted as the Pre-treatment Solution.

### (3) Quantitative Determination of Recovered Nucleic Acid

The total yield of DNA purified by the method described above, and the height of foam (a measurement of the length of the foam generated from the opening upon discharging the sample solution), are shown in the following Table 1 (*next page*).

Thus, it is clear that when the above unit for separation and purification of nucleic acid is used, it is easy to separate and purify nucleic acids. Furthermore, from the data disclosed in Table 1, it is also clear that the amount of DNA obtained from the Pre-treatment Solution of the presently claimed invention, to which an acetylene glycol type of antifoaming agent was added, or to which an antifoaming agent of a mixture of acetylene glycol and silicone oil was added, was able to be maintained and, marked and unexpected suppression of foaming was observed. The degree of suppression of foam by the anti-foaming agent in the Pre-Treatment Solution of the presently claimed invention would not have been expected by one of ordinary skill in the art.

Trade Name	Ingredient	Manufacturer	Foam Height	Yield of DNA
(nothing used)			x	100% (Standard Value)
TSA 770	Oil compound type silicone	Momentive Performance Materials Inc.	Δ	86%
TSA 732	Emulsion type silicone	Momentive Performance Materials Inc.	Δ	81%
TSA 7341	MIX type silicone	Momentive Performance Materials Inc.	Δ	88%
Disfoam BF-75	Polyalkylene glycol derivative	Nippon Yushi Co., Ltd.	Δ	89%
Disfoam BF-7	Polyalkylene glycol derivative	Nippon Yushi Co., Ltd.	Δ	89%
Disfoam CC-218	Polyalkylene glycol derivative	Nippon Yushi Co., Ltd.	Δ	81%
Disfoam CC-118V	Polyalkylene glycol derivative	Nippon Yushi Co., Ltd.	Δ	72%
Disfoam CC-118	Polyalkylene glycol derivative	Nippon Yushi Co., Ltd.	Δ	78%
Emulgen 404	Polyoxyethylene oleyl ether	Kao Corporation	Δ	78%
Excel T95	Glycerol fatty acid ester	Kao Corporation	Δ	84%
Antifoaming Agent No. 1	Poly ether	Kao Corporation	Δ	88%
Defoamer No. 1	Polyether	FUJIFILM Corporation	Δ	82%
Surfinol 1041	Acetylene glycol	Nissin Chemical Industry Co., Ltd.	O	103%
Surfinol DF110D	Acetylene glycol	Nissin Chemical Industry Co., Ltd.	O	99%
Olfine SPC	Acetylene glycol	Nissin Chemical Industry Co., Ltd.	O	98%
Olfine AK-02	Acetylene glycol + silicon	Nissin Chemical Industry Co., Ltd.	OO	96%
Olfine AF-103	Acetylene glycol	Nissin Chemical Industry Co., Ltd.	O	102%

x: Foam size was not smaller than 50 mm

Δ: Foam size was 30 to 50 mm

O: Foam size was 10 to 30 mm

OO: Foam size was not larger than 10 mm

Application No. 10/568,101

Docket No.: 0649-1217PUS1

STATEMENT UNDER 18 U.S.C. § 1001

I hereby declare that all statements made herein of any own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 06/11/2008

Toshihiro Mori

Toshihiro Mori